

(c) *Preparation of the spotting solutions.* Prepare a solution of the sample containing 25 milligrams per milliliter of cephalexin hydrochloride in water. Prepare a solution of cephalexin monohydrate reference material at a concentration of 25 milligrams per milliliter. Add water and 0.1*N* hydrochloric acid in a dropwise mode until the material is completely dissolved.

(d) *Procedure.* Pour the developing solvent into the glass trough at the bottom of the chromatography tank. Cover and seal the tank. Allow it to equilibrate for 1 hour. Prepare a plate as follows: On a line 2 centimeters from the base of the plate, and at intervals of 2 centimeters, spot approximately 5 microliters of the standard solution to points 1 and 3 and approximately 5 microliters of the sample solution to point 2. After all spots are thoroughly dry, place the plate directly into the glass trough of the chromatography tank. Cover and seal the tank. Allow the solvent front to travel approximately 15 centimeters from the starting line. Remove the plate from the tank and allow it to air dry.

(e) *Evaluation.* View the dry plate under ultraviolet light (254 nanometers). Measure the distance the solvent front traveled from the starting line and the distance the spots are from the starting line. Calculate the  $R_f$  value by dividing the latter by the former. The sample and standard should have spots of corresponding  $R_f$  values of approximately 0.35.

[54 FR 48860, Nov. 28, 1989; 54 FR 51816, Dec. 18, 1989]

**§ 436.368 Thin layer chromatographic identity test for cefprozil.**

(a) *Equipment*—(1) *Chromatography tank.* Use a glass rectangular tank approximately 23 x 23 x 9 centimeters lined with filter paper and equipped with a tight-fitting cover.

(2) *Plates.* Use 20 x 20 centimeter thin layer chromatography plates coated with silica gel GF to a thickness of 250 microns.

(b) *Reagents*—(1) *Diluent.* Mix 0.1*N* HCl and acetone in volumetric proportions of 1:4.

(2) *Developing solvent.* Mix n-butanol, glacial acetic acid and water in volumetric proportions of 60:20:20.

(3) *Detection reagent.* Iodine vapor.

(c) *Assay solutions*—(1) *Reference standard solution.* Dissolve 50 milligrams of cefprozil (Z) reference standard in 10 milliliters of diluent.

(2) *Sample solution.* Place an amount of sample containing approximately 50 milligrams of cefprozil in a 20-milliliter glass stoppered vial. Add 10 milliliters of diluent. Shake for 5 minutes and allow the solids to settle.

(d) *Procedure.* Pour a suitable quantity of the developing solvent into a glass, chromatographic tank lined with filter paper and allow to equilibrate for 1 hour. On a line 2 centimeters from the bottom edge of the plate, spot 10 microliters each of the reference solution and sample solution. Draw a line indicating the distance to which the developing solvent must travel at a point 12 centimeters from the bottom edge of the plate. Place the plate in the tank and allow the solvent to migrate to the finishing line. Remove the plate and air dry in a fume hood. Place the dried plate in a chamber containing iodine vapors.

(e) *Evaluation.* Measure the distance the solvent front traveled from the starting line, and the distance the spots are from the starting line. Divide the latter by the former to calculate the  $R_f$  value. The identity test is positive if the sample solution produces a yellow spot at the same  $R_f$  value and has the same appearance as the spot obtained for the reference solution. The  $R_f$  value for cefprozil (Z) is approximately 0.45. Cefprozil (E), has an  $R_f$  value of approximately 0.47. Cefprozil (Z) is "absent" if the above test is performed and no spots, which correspond to those from the reference solution, are obtained for the sample.

[58 FR 26660, May 4, 1993]

**§ 436.369 Thin layer chromatography test for free *N*-isobutylpiperidone content in rifabutin.**

(a) *Equipment*—(1) *Chromatography tank.* A rectangular tank, approximately 23 X 23 X 9 centimeters, with a glass solvent trough on the bottom and a tight-fitting cover.

(2) *Iodine vapor chamber.* A rectangular tank, approximately 23 X 23 X 9 centimeters, with a suitable cover, containing iodine crystals.

(3) *Plates.* Use 20 X 20 centimeter thin layer chromatography plates coated with silica gel 60F 254 or equivalent to a thickness of 250 microns.

(b) *Reagents*—(1) *Developing solvent.* Mix petroleum ether (b.p. 60 to 80 ° C) and acetone in volumetric proportions of 100:30, respectively.

(2) *Spray solution.* Prepare a 1 percent solution of soluble starch in water (containing 0.01 percent mercuric iodide).

(c) *Preparation of spotting solutions*—(1) *Sample solution.* Prepare a solution of the rifabutin sample in 1:1 chloroform/methanol to contain 10 milligrams per milliliter.

(2) *Standard solution.* Prepare a solution of *N*-isobutylpiperidone standard in 1:1 chloroform/methanol to contain 1 milligram per milliliter. Transfer aliquots of 0.5, 1.0, 2.0, 5.0, and 10.0 milliliters into separate 100-milliliter volumetric flasks and dilute to volume with 1:1 chloroform/methanol. These solutions contain, respectively, the equivalent of 0.05, 0.1, 0.2, 0.5, and 1.0 percent of *N*-isobutylpiperidone.

(d) *Procedure.* Pour 100 milliliters of developing solvent into the glass trough on the bottom of the unlined chromatography tank. Cover and seal the tank. Allow it to equilibrate while the plate is being prepared. Prepare a plate as follows: on a line 2.0 centimeters from the base of the thin layer chromatography plate, and at intervals of 2.0 centimeters, apply 10 microliters of each of the standard solutions and the sample solution prepared as directed above. After the spots are thoroughly dry, place the plate into the trough in the bottom of the tank. Cover and tightly seal the tank, allow the solvent front to travel about 15 centimeters from the starting line and then remove the plate from the tank. Air dry the plate. Warm the iodine vapor chamber to vaporize the iodine crystals and place the dry plate in the iodine vapor chamber until the spots are visible (usually about 5 minutes). Remove the plate from the iodine vapor chamber and spray with 1 percent starch solution.

(e) *Evaluation.* Measure the distance the solvent front traveled from the starting line and the distance the spots are from the starting line. Calculate

the  $R_f$  value by dividing the latter by the former. *N*-isobutylpiperidone has an  $R_f$  value of about 0.3. Rifabutin has an  $R_f$  value of about 0.1. Compare the size and intensity of any *N*-isobutylpiperidone spots in the sample lane with the *N*-isobutylpiperidone spots in the standard lanes, and report the percentage of *N*-isobutylpiperidone in the sample.

[59 FR 40806, Aug. 10, 1994]

#### § 436.370 Spectrophotometric identity test for rifabutin capsules.

(a) *Equipment.* A suitable spectrophotometer capable of recording the ultraviolet spectrum in the 200 to 400 nanometer range, using suitable quartz cells of 1 centimeter pathlength.

(b) *Preparation of working standard and sample solution*—(1) *Working standard solution.* Suspend approximately 200 milligrams of rifabutin working standard in 20 milliliters of methanol and sonicate for approximately 5 minutes. Filter the resulting solution through a suitable 0.5 micrometer filter. Transfer a 2-milliliter aliquot of the filtered solution to a 100-milliliter volumetric flask and fill to volume with methanol. Further dilute with methanol to obtain a solution containing 20 micrograms of rifabutin activity per milliliter.

(2) *Sample solution.* Empty and combine the contents of five capsules. Suspend a quantity of the capsule contents equivalent to 200 milligrams of rifabutin in 20 milliliters of methanol. Sonicate for about 5 minutes and then filter through an appropriate 0.5 micrometer filter. Transfer a 2-milliliter aliquot to a 100-milliliter volumetric flask and dilute to volume with methanol. Further dilute with methanol to obtain a solution containing 20 micrograms of rifabutin activity per milliliter (estimated).

(c) *Procedure.* Using a suitable spectrophotometer equipped with 1.0 centimeter cells and methanol as the blank, determine the absorbance spectra of the working standard and sample solutions over the ultraviolet range of 250 to 300 nanometers.

(d) *Evaluation.* Compare the spectrum of the sample to that of the working standard. The identity of the rifabutin capsules is confirmed by quantitative comparison of the two spectra with an